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(54) Title: REGULATOR REGIONS OF G PROTEINS (57) Abstract A molecule having a peptide sequence of 80 or fewer amino acid residues containing the amino acid sequence of an anticouplone of any of the G proteins, which molecule is useful for inhibiting activation of the G protein by its G-coupled receptor.		



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REGULATOR REGIONS OF G PROTEINS

The field of the invention is cell receptor-based signal transduction.

5

Background of the Invention

Guanine nucleotide-binding proteins (G proteins) are a family of signal transducers which are found associated with the plasma membrane within eukaryotic cells, and which act to relay signals from certain cell-surface receptor molecules (G-coupled receptors) to effectors in the plasma membrane. G proteins are functional dimers consisting of α ($G\alpha$) and $\beta\gamma$ ($G\beta\gamma$) subunits (Stryer et al., Ann. Rev. Cell Biol. 2:391-419, 1986). Some presently-known members of this family include the G proteins termed G_{o1} , G_{o2} , G_{i1} , G_{i2} , G_{i3} , G_s , G_q , and the G_q -related family which presently includes G_{11} , G_{12} , G_{13} , G_{14} , G_{15} , and G_{16} (see, e.g., Kaziro, "Structures of the Genes Coding for the α Subunits of G Proteins", Chapter 11 in ADP-ribosylating Toxins and G Proteins (Moss, J., and Vaughan, M. eds.) pp189-206, American Society for Microbiology, Washington, D.C., 1988). Each individual G protein is characterized as such by the particular α subunit species (e.g., $G_{o1}\alpha$, $G_{o2}\alpha$, etc.) which forms the α portion of the dimeric protein. In their unstimulated condition, G proteins are $\alpha\beta\gamma$ forms bound to guanosine diphosphate (GDP); upon receptor stimulation they dissociate into GTP-bound $G\alpha$ and nucleotide-free $G\beta\gamma$, leading to the activation of the next effector protein in the cascade. In at least one case, this activation results in the opening of a calcium channel in the membrane, thereby increasing calcium influx in the cell (Kojima et al., Biochem. Biophys. Res. Commun. 154:9-19, 1988; Matsunaga et al., Am. J. Physiol. 255:C442-C446, 1988).

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Okamoto et al. (Cell 62:709-717, 1990; hereby incorporated by reference) identified the region of one G-coupled receptor protein (insulin-like growth factor II receptor, or IGF-IIR) which directly interacts with and
5 activates G_{i2} ; this region of IGF-IIR is sometimes referred to as that receptor's "couplone". A synthetic peptide (termed "Peptide 14") having a sequence identical to that of IGF-IIR's couplone was found to bind to and activate G_{i2} . Studies on synthetic variants of this
10 peptide permitted Okamoto et al. to posit a critical peptide length (10 to 17 residues) and a motif for the couplone: two B's at the amino terminus of the peptide, and B-B-X-B or B-B-X-X-B at the carboxy terminus, where B is a basic amino acid (Lys, Arg, or His) and X is any
15 nonbasic amino acid. In another G-coupled receptor, β_2 -adrenergic receptor (β_2 AR), a G_s -activator region termed β III-2 has been identified in the third intracellular loop of β_2 AR (Okamoto et al., 1991a).

Summary of the Invention

20 Applicants have identified the precise region on each of the known eukaryotic G proteins which interacts directly with the associated G-coupled receptor proteins. This region, termed the "anticouplone" or regulator region of the G protein, has been found to be critical to
25 the function of the G protein, and therefore to signal transduction by the associated receptor. The invention thus encompasses a molecule of 80 or fewer amino acid residues (preferably 50 or fewer, more preferably 40 or fewer, and most preferably 30 or fewer) containing the
30 amino acid sequence of an anticouplone of any of the G proteins (e.g., a mammalian G protein such as G_{o1} , G_{o2} , G_{i1} , G_{i2} , G_{i3} , G_q , and G_s). The anticouplone region of a given G protein is located near the carboxy terminus of the G protein, from the 4th residue from the carboxy

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terminus at least to the 18th residue from that terminus, inclusive, and preferably to approximately the 27th residue from that terminus, inclusive. The molecule may contain an amino acid sequence defined by

5 DXXXXVIIKNNLXXC (SEQ ID NO: 1),

where X denotes any amino acid (up to two of which may be deleted) and the other letters represent specific amino acids according to the standard single-letter amino acid code. More preferably, the peptide sequence is defined

10 by

 DAVTXVIIKNNLKXC (SEQ ID NO: 2).

Specific examples of such a sequence include

 DAVTDVIIKNNLKDC (SEQ ID NO: 3),

 DTKNVQFVFDVTDVIIKNNLKDC (SEQ ID NO: 4),

15 DAVTDVIIKNNLKEC (SEQ ID NO: 5), and

 DTKNVQFVFDVTDVIIKNNLKEC (SEQ ID NO: 6).

Another version of the molecule of the invention includes the sequence

 DAVTXIIIIAXNLRXC (SEQ ID NO: 7);

20 examples include

 DAVTDIIIIANNLRGC (SEQ ID NO: 8),

 NNIQVVFDVTDIIIIANNLRGC (SEQ ID NO: 9),

 DAVTDIIIIAKNLRGC (SEQ ID NO: 10), or

 NNIQFVFDVTDIIIIAKNLRGC (SEQ ID NO: 11).

25 Other molecules of the invention include a sequence

 NDCRDIIQRMHLRQY (SEQ ID NO: 12),

 DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13),

 AAVKDTILQLNLKEY (SEQ ID NO: 14), or

 DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).

30 The molecules of the invention may be employed in a method of inhibiting activation of a given G protein by a G-coupled receptor on a cell, which method includes the step of contacting the cell with, or otherwise introducing into the cell, a molecule containing the

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anticouplone sequence of the G protein, which molecule contains 40 or fewer amino acid residues. This method may be carried out, for example, by local or systemic administration of the appropriate peptide or modified peptide, or by introducing into the cell a nucleic acid encoding the anticouplone peptide together with all promoters and other elements necessary for expression of the peptide from the nucleic acid. Techniques for constructing such a nucleic acid and introducing it into the target cells are well known to those of ordinary skill in the art. Where the G protein of interest is G_{i1} , G_{i2} , or G_{i3} , the targeted receptor may be, for example, insulin-like growth factor II receptor (IGF-IIR), muscarinic acetylcholine receptor (mAChR), somatostatin receptor, D_2 -dopamine receptor, α_2 -adrenergic receptor, adenosine receptor, thrombin receptor, $TGF\beta$ receptor, or any other receptor which is functionally coupled to one of those G proteins. Where the G protein of interest is G_{o1} or G_{o2} , the receptor may be, for example, γ -butyric acid (GABA) receptor, somatostatin receptor, muscarinic acetylcholine receptor (mAChR), α_2 -adrenergic receptor, adenosine receptor, thrombin receptor, or $TGF\beta$ receptor. Examples of G_s -linked receptors include β_2 -adrenergic receptor, glucagon receptor, IL-1 receptor, and D_1 -dopamine receptor; while examples of G_q -linked receptors include the PTH/PTHrP receptor, calcitonin receptor, endothelin receptor, T cell receptor, angiotensin receptor, platelet activating factor receptor, and thromboxane A_2 receptor.

Other features and advantages of the invention will be apparent from the detailed description set forth below, and from the claims.

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Brief Description of the Drawings

Figs. 1a-1d is a set of graphs illustrating the effect of ACG_{i2} on G protein activation. (A) Effects of ACG_{i2} and control peptides on peptide 14-induced G_{i2} activation. (B) Effect of ACG_{i2} on TrM-peptide 14-induced G_{i2} activation and $\beta III-2$ -induced G_s activation. (C) Effect of ACG_{i2} on G_{i1} and G_{i3} activation. (D) Effect of ACG_{i2} on MIII-induced G_{i2} and G_o activation.

Figs. 2a-2b is a pair of graphs illustrating the mode of action of ACG_{i2} . (A) Dose/response relationship of peptide 14 action in the presence of various concentrations of ACG_{i2} . (B) Effect of ACG_{i2} on activation of 1 nM G_{i2} induced by peptide 14.

Figs. 3a-3c is a set of graphs illustrating the structure-function relationship of ACG_{i2} on peptide 14-induced G_{i2} activation. (A) Effect of ACG_{i2} and high potency group variants. (B) Effect of low potency group variants. (C) Effect of intermediate potency group variants.

Fig. 4 is a bar graph illustrating the effect of ACG_{i2} on mAChR- G_{i2} coupling.

Fig. 5 is a schematic drawing illustrating a proposed mechanism whereby the activation state of G_{i2} is affected by peptide 14 and/or ACG_{i2} peptide.

Fig. 6 is a set of three photomicrographs illustrating the inhibitory effect of ACG_{i2} on serum-induced CHO cell growth.

Fig. 7 is an autoradiograph of an SDS-PAGE analysis illustrating the effect of ACG_{O2} on TGF β -induced fibronectin secretion from kidney cells.

Fig. 8 is a pair of photomicrographs illustrating the effect of ACG_{i2} on cardiac fibrosis in myopathic hamsters.

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Detailed Description

The anticouplones of the invention were discovered by analyzing the sequences of the known G proteins to identify the region on each which was deemed likely to function as an anticouplone, and then testing each candidate anticouplone, and variants thereof, in appropriate *in vitro* and *in vivo* assays.

Identification of Candidate Anticouplone Sequences

On the assumption that, in order to account for the specificity of each receptor for a given type of G protein, the $G\alpha$ subunit of a given G protein must participate in the direct interaction with the associated receptor's couplone, it was postulated that the anticouplone, or signal-acceptor domain, of the G protein dimer would be located, at least in part, on the $G\alpha$ subunit. Certain criteria, described below, were then established to define the putative signal-acceptor domain on each G protein's α subunit. These criteria were initially applied to determine the anticouplone of $G_{12}\alpha$, as follows: Although a recent publication suggested otherwise (Okuma et al., J. Biol. Chem. 267:14826-14831, 1992), a number of lines of evidence indicated to Applicant that at least a portion of the anticouplone sequence would be located at or near the carboxy-terminal end of the G protein (Okamoto et al., Proc. Natl. Acad. Sci. USA 88:3020-8023, 1991; Ui et al., Adv. Cyclic Nucleotide Protein Phosphorylation Res., 17:145-151, 1984; Rall et al., FEBS Lett. 224:365-371, 1987; Masters et al., Science 241:448-451, 1988; Moss and Vaughan, eds., American Society for Microbiology, 1988, pp.207-224; Simonds et al., Proc. Natl. Acad. Sci. USA, 86:7809-7813, 1989; McClue et al., FEBS Lett. 269:430-434, 1990; Shenker et al., J. Biol. Chem. 266:9309-9313, 1991; Murray-Whelan et al., J. Biol. Chem. 267:2960-2965, 1992; Meinkoth et al., J. Biol. Chem. 267:13239-13245, 1992;

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Okamoto and Nishimoto, Proc. Natl. Acad. Sci. USA
88:8020-8023, 1991; and Sullivan et al., Nature 330:758-
760, 1987). Therefore, the first criterion specified
that the candidate signal-acceptor region be located in
5 the carboxy-terminal portion of $G_{12}\alpha$.

Within the carboxy-terminal portion of $G_{12}\alpha$,
Applicant focused on the region Asp338-Asp351 (SEQ ID NO:
16). This region has a primary structure in which the
negatively charged residues are oriented as matching the
10 positively charged basic residues essential for the G_{12} -
activating function of Peptide 14. The structural
determinants for this function of Peptide 14 were
hypothesized to be (i) two basic residues at its amino
terminus and (ii) a carboxy terminal motif of B-B-X-B or
15 B-B-X-X-B. Asp338-Asp351 (DAVTDVVIKNNLKD) (SEQ ID NO:
16) is the only region of G_{12} that meets all of these
criteria. This sequence was therefore tentatively
identified as the $G_{12}\alpha$ anticouplone. It was found that
 $G_{11}\alpha$ contains the same 14 residues at a corresponding
20 region, while $G_{13}\alpha$ contains the almost identical sequence
DAVTDVVIKNNLKE (SEQ ID NO: 17), in which the N-terminal
Asp is replaced by Glu. When the four criteria are
applied to $G_{01}\alpha$ and $G_{02}\alpha$, the resulting anticouplone
sequences are defined by DAVTDIIIANNLRGC (SEQ ID NO: 8)
25 and DAVTDIIIAKNLRGC (SEQ ID NO: 10), respectively. The
anticouplone sequence of G_s is NDCRDIIQRMHLRQY (SEQ ID
NO: 12), while that of G_q is AAVKDTILQLNLKEY (SEQ ID NO:
14).

The above reasoning was used to identify a
30 putative receptor-binding domain on each individual $G\alpha$
species. Later experiments carried out with synthetic
peptides representing these putative receptor-binding
domains have suggested that, rather than receptor-binding
domains, these particular regions of the $G\alpha$ polypeptides
35 may serve as $G\alpha$ -activating regulator regions. This

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completely unexpected finding is discussed in detail below.

Synthesis of Anticouplone Peptides

The peptides used in this study were synthesized and purified as described (Okamoto et al., 1990; Okamoto and Nishimoto, 1991; Okamoto et al., 1991a; Okamoto and Nishimoto, 1992). The following peptides were prepared:

1. ACG_{i2}, corresponding both to G_{i1}α Asp337-Cys351 and to G_{i2}α Asp338-Cys352 (DAVTDVVIKNNLKDC; SEQ ID NO: 3). The fact that pertussis toxin, which catalyzes ADP-ribosylation of Cys352, attenuates G_{i2} activation produced by peptide 14 (Okamoto et al., 1990, *supra*) as well as by IGF-IIR (Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989), prompted the inclusion of Cys352 in this peptide.
2. ACG_{i3} (DAVTDVVIKNNLKEC; SEQ ID NO: 5).
3. ACG_{o1} (DAVTDIIIANNLRGC; SEQ ID NO: 8).
4. ACG_{o2} (DAVTDIIIAKNLRGC; SEQ ID NO: 10).
5. ACG_s (NDCRDIIQRMHLRQY; SEQ ID NO: 12).
6. ACG_q (AAVKDTILQLNLKEY; SEQ ID NO: 14).
7. Alternative G_{o1} peptide
NNIQVVFDAVTDIIIANNLRGC (SEQ ID NO: 9).
8. Alternative G_{o2} peptide NNIQFVFDAVTDIIIAKNLRGC (SEQ ID NO: 11).
9. Alternative G_s peptide
DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13).
10. Alternative G_q peptide
DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).
11. Control peptide Glu297-Asp310
(EAASYIQSKFEKLN; SEQ ID NO: 18) corresponding to G_{i2}α Glu297-Asp310.
12. Control peptide Lys346-Phe355 (KNNLKDCGLF; SEQ ID NO: 19), representing the carboxy-terminal 10 residues of G_{i2}α.
13. A₁ variant (AAVTDVVIKNNLKDC; SEQ ID NO: 20).

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14. A₂ variant (DAVTAVIIKNNLKDC; SEQ ID NO: 21).
15. A₃ variant (DAVTDVIIKANLKDC; SEQ ID NO: 22).
16. A₄ variant (DAVTDVIIKNALKDC; SEQ ID NO: 23).
17. A₅ variant (DAVTDVIIKNNLKAC; SEQ ID NO: 24).
5 18. p9 variant (DAVTDVIIK; SEQ ID NO: 25).
19. p11 variant (DVIIKNNLKDC; SEQ ID NO: 26).
20. del C variant (DAVTDVIIKNNLKDC; SEQ ID NO:
16).
21. Peptide 14 (RVGLVRGEKARKGK; SEQ ID NO: 27),
10 the 14-residue couplone portion of the G_{i2}-coupled
receptor protein IGF-IIR.
22. TrM peptide 14 (LTACLLTLLLYRVGLVRGEKARKGK;
SEQ ID NO: 28), peptide 14 linked to the native
transmembrane domain of IGF-IIR.
15 23. β III-2 (RRSSKFCLKEHKALK; SEQ ID NO: 29), the
15-residue couplone region of the G_s-coupled receptor
 β 2AR.
24. MIII peptide (RNQVRKKRQMAARERKVTR; SEQ ID NO:
30), the 19-residue couplone region of the G_i/G_o-coupled
20 M₄ muscarinic acetylcholine receptor (M₄AChR).

Designed but not prepared were two additional
peptides in the series:

25. Alternative G_{i1}/G_{i2} peptide
DTKNVQFVFDVAVTDVIIKNNLKDC (SEQ ID NO: 4), and
25 26. Alternative G_{i3} peptide
DTKNVQFVFDVAVTDVIIKNNLKEC (SEQ ID NO: 6).

In Vitro, Cell-free Assays of Anticouplone Peptides **Materials and Methods**

The *in vitro* assays described below utilize the
30 couplone portion of each of a number of G-coupled
receptors, instead of an intact receptor molecule, as a
means for activating the corresponding anticouplone or
intact G-protein. Except as noted below, G_{i2} used in this

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study was trimeric G_{i2} (i.e., $G_{i2}\alpha\beta\gamma$) purified from bovine brain to near homogeneity (Katada et al., *FEBS Lett.* 213:353-358 (1987), stored in buffer A (20 mM hepes/NaOH (pH 7.4), 1 mM EDTA, and 0.7% CHAPS), and diluted ≥ 10 fold for assays. The G_{i2} used for the reconstitution experiment was trimeric G_{i2} purified from bovine spleen to homogeneity and dissolved in buffer B (Morishita et al., 1989). $G_{i3}\alpha$ (Morishita et al., *Biochem. Biophys. Res. Commun.* 172:249-255 (1990), was purified from bovine spleen and used in combination with 1.5-fold concentrated $G\beta\gamma$ (Katada et al., 1987). This preparation of $G_{i3}\alpha$ was 10-20% contaminated by $G_{i1}\alpha$, but not by other G proteins. Trimeric G_s and $G\beta\gamma$ purified from bovine brain to near homogeneity (Katada et al., 1987) were also stored in buffer A.

mAChR (muscarinic acetylcholine receptor) was purified from porcine brain to near homogeneity (Haga et al., 1986) and dissolved in 0.5 M potassium phosphate buffer (pH 7.0) containing 0.1% digitonin. This receptor preparation was reconstituted with G_{i2} in phospholipid vesicles using a gel filtration method as described previously (Nishimoto et al., 1989). The concentrations of mAChR and G_{i2} in the vesicles were 6.9 nM and 29 nM, respectively.

GTP γ S binding Assay - GTP γ S binding to G proteins was assayed at 37°C in 20 mM Hepes/NaOH buffer (pH 7.4) containing 130 μ M $MgCl_2$, 110 μ M EDTA and 60 nM [35 S]GTP γ S, as described previously (Okamoto et al., 1990). GTP γ S binding to polypeptides was negligible. The total amount of G protein was measured as maximal GTP γ S binding at room temperature, as described (Okamoto et al., *Cell* 67:723-730, 1991a). [35 S]GTP γ S was purchased from DuPont-New England Nuclear. Binding of GTP γ S to G proteins obeyed first-order kinetics according to the equation $\ln[(BT - B)/B_T] = -k_{app}t$, where B is the binding at time t

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and B_T is the total binding observable at an infinite time. Thus, the apparent first-order rate constant for GTP γ S binding (k_{app}), which is equal to the slope of the tangent to the GTP γ S binding curve at time 0 and
5 represents the actual GTP γ S binding rate, was calculated from this equation.

Results

In the experiment illustrated in Fig. 1A, 10nM G_{i2} was incubated with 30 μ M peptide 14 (RVGLVRGEKARKGK; SEQ ID NO: 27), the couplone portion of the IGF-II receptor, in the presence of various concentrations of (\square) ACG $_{i2}$ (DAVTDVVIKNNLKDC; SEQ ID NO: 3), (\diamond) the control $G_{i2}\alpha$ Glu297-Asp310 peptide (EAASYIQSKFEKLN; SEQ ID NO: 18), or (\diamond) the control $G_{i2}\alpha$ Lys346-Phe355 peptide (KNNLKDCGLF; SEQ ID NO: 19) for 5 min under the GTP γ S binding assay conditions described above, and GTP γ S binding to G proteins was measured. The effect of ACG $_{i2}$ on G_{i2} in the absence of peptide 14 is indicated by \square . Percent stimulation of GTP γ S binding was calculated by
20 subtracting the basal fractional binding and dividing by the stimulated fractional binding observed in the absence of ACG $_{i2}$. The basal fractional binding (= 0%) was 0.39 mol GTP γ S binding/mol G_i /5 min (5 min^{-1}). The 100% increase represents the GTP γ S binding of 0.85 (5 min^{-1}).
25 Values in all figures represent the mean \pm S.E. of three experiments.

Fig. 1A shows that ACG $_{i2}$ peptide at 30 μ M inhibits binding of GTP γ S to G_{i2} in the presence of peptide 14 to the basal level of binding exhibited by G_{i2} in the absence
30 of peptide 14. The IC_{50} was $\approx 3\mu$ M. ACG $_{i2}$ had no effect on GTP γ S binding to G_{i2} in the absence of peptide 14. $G_{i2}\alpha$ has another region that satisfies (I)-(III): residues Glu297-Asn310 (EAASYIQSKFEKLN; SEQ ID NO: 18). This peptide, however, showed no effect on peptide 14-induced

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G_{i2} activation. As another control, a peptide representing the carboxy-terminal 10 residues of $G_{i2}\alpha$ (KNNLKDCGLF; SEQ ID NO: 19) was synthesized and tested in this assay. Unlike ACG_{i2} , this decapeptide exhibited no inhibitory effect on peptide 14-induced G_{i2} activation. These findings suggest that the antagonizing effect of ACG_{i2} on G_{i2} activation is attributable to the specific amino acid sequence of the peptide.

Transmembrane domain-connected peptide 14 ["TrM-peptide 14"; LTACLLTLLLYRVGLVRGEKARKGK (SEQ ID NO: 28)] is peptide 14 that is linked to the native transmembrane domain of IGF-IIR. It has been demonstrated that, in phospholipid vesicles, TrM-peptide 14 can interact with G_{i2} in a manner more similar to that of the native holo receptor than can peptide 14 (Okamoto et al., 1990). In one of the experiments shown in Fig.1B, 0.3 nM G_{i2} reconstituted in phospholipid vesicles with 100nM TrM-peptide 14 was exposed to various concentrations of ACG_{i2} , and GTP γ S binding was measured (\diamond). The basal fractional GTP γ S binding to G_{i2} in this experiment was 0.39 (5 min⁻¹), and the 100% binding level was 0.88 (5 min⁻¹). ACG_{i2} completely blocked the stimulatory effect of 100 nM TrM-peptide 14, with an IC₅₀ of 3 μ M (Fig. 1B). Thus, even where the context more closely approximates the native environment of the G protein than in the experiment shown in Fig.1A, ACG_{i2} effectively blocks the stimulatory effect of the couplone on G_{i2} with the same potency and efficacy as observed in the latter experiment.

The effect of ACG_{i2} on β III-2-induced G_s activation is shown in Fig.1B (\square). GTP γ S binding was measured following incubation of solubilized G_s at 10nM with 1 μ M β III-2 (RRSSKFCLKEHKALK; SEQ ID NO: 29) in the presence of ACG_{i2} for 10 min. The basal fractional GTP γ S binding to G_s was 0.30 (10 min⁻¹), and 100% binding was 0.60 (10 min⁻¹). ACG_{i2} had no effect at 1 μ M. Unlike

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inhibition of stimulation of G_{i2} , which reaches 100% at $3\mu\text{M}$ ACG_{i2} , the maximal inhibition of $\beta\text{III-2}$ -stimulated G_s by ACG_{i2} was only 40%, reflecting the fact that the G_s anticouplone is not identical to ACG_{i2} , and so the latter peptide binds to the G_s -coupled receptor relatively inefficiently. At higher concentrations, ACG_{i2} proved to have a positive rather than inhibitory effect on G_s stimulation.

The region of $G_s\alpha$ that is comparable to the ACG_{i2} region of $G_{i2}\alpha$ in topographical structure was synthesized; this region is defined by Asn384-Tyr398 of $G_s\alpha$ (NDCRDIIQRMHLRQY; SEQ ID NO: 12). At concentrations ranging up to $100\mu\text{M}$, this peptide (ACG_s) had no effect at all on peptide 14-induced G_{i2} activation, again reflecting the specificity of each anticouplone (data not shown).

Peptide 14 is capable of activating G_{i1} and G_{i3} as well as G_{i2} (Okamoto et al., 1990). The effect of ACG_{i2} on peptide 14-induced activation of these G_i proteins was examined as follows: 10 nM G_{i1} (\square) or G_{i3} (\diamond) was incubated with $30\mu\text{M}$ peptide 14 in the presence of various concentrations of ACG_{i2} for 4 min, and $\text{GTP}\gamma\text{S}$ binding was measured. The fractional $\text{GTP}\gamma\text{S}$ binding to G_{i1} or G_{i3} at 0% was (in 4 min^{-1}) 0.38 or 0.30, and that at 100% was 0.54 or 0.63, respectively. Surprisingly, ACG_{i2} had relatively little effect on G_{i1} activation (Fig. 1C). Even high concentrations ($\geq 30\mu\text{M}$) of ACG_{i2} maximally inhibited G_{i1} activation by only 30%. The G_{i1} preparation used had trace contamination by G_o , as described (Katada et al., *FEBS Lett.* 213:353-358 (1987). Since the region of $G_{i1}\alpha$ comparable to ACG_{i2} has a sequence identical to ACG_{i2} , as mentioned above, it appears this region on $G_{i1}\alpha$ does not possess the same function as the identical region on $G_{i2}\alpha$.

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Fig. 1C also shows the effect of ACG_{i2} on peptide 14-induced G_{i3} activation. ACG_{i2} maximally inhibited G_{i3} activation by $\approx 60\%$. This suggests that ACG_{i2} has an antagonizing effect on G_i protein activation in the order of $G_{i2} > G_{i3} > G_{i1}$. These results indicate that the ACG_{i2} peptide blocks G_{i2} activation in a selective manner.

The MIII peptide is a G_i/G_o -activator region in the third intracellular loop of the G-coupled receptor termed M_4AChR (Okamoto et al., J. Biol. Chem. 267:8342-8346, 1992). This peptide has the sequence RNQVRKKRQMAARERKVTR (SEQ ID NO: 30). In the experiments illustrated in Fig. 1D, 10 nM G_o (\square) or G_{i2} (\diamond) was incubated with 1 μM MIII for 2 or 5 min, respectively, and GTP γ S binding was measured in the same manner as in Fig. 1A. The fractional GTP γ S binding to G_o at 0% and 100% was 0.36 and 0.86 (2 min $^{-1}$), respectively, and that to G_{i2} at 0% and 100% was 0.30 and 0.96 (5 min $^{-1}$), respectively. MIII-induced G_{i2} activation was found to be attenuated by ACG_{i2} in a dose-dependent manner, with an IC_{50} of 10 μM (Fig. 1D). ACG_{i2} can therefore inhibit G_{i2} activation caused by the effector peptides of m_4AChR and IGF-IIR with similar potencies. In contrast, ACG_{i2} had no effect on MIII-induced G_o activation at $\leq 10 \mu M$, and its effect on MIII-induced G_o activation at higher concentrations ($IC_{50} \approx 30 \mu M$) was significantly (≈ 3 times) less potent than its effect on MIII-induced G_{i2} activation. This provides strong evidence that ACG_{i2} acts on G proteins rather than on receptor peptides.

To verify the notion that ACG_{i2} acts on G_{i2} itself, and not on receptor sequences, two series of experiments were performed. First, the dose-response curve for peptide 14 action on G_{i2} in the presence of various concentrations of ACG_{i2} was generated (Fig. 2A). Following incubation of 10 nM G_{i2} with various concentrations of peptide 14 in the absence (\square) or

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presence of 10 μM (\diamond) or 30 μM (\square) ACG_{12} for 5 min, $\text{GTP}\gamma\text{S}$ binding was assayed; fractional $\text{GTP}\gamma\text{S}$ binding to G_{12} is indicated in the figure. As the concentration of ACG_{12} increased, the efficacy of peptide 14 action was found to be decreased. This suggests that ACG_{12} does not compete with G_{12} for peptide 14.

The second series of experiments investigated the effect of ACG_{12} on lower concentrations of G_{12} that had been activated by peptide 14 to the same extent (Fig. 2B). 1nM G_{12} was incubated for 5 min with 3 μM peptide 14 in the presence of various concentrations of ACG_{12} , and $\text{GTP}\gamma\text{S}$ binding was measured. The fractional $\text{GTP}\gamma\text{S}$ binding at 0% and 100% in this experiment was 0.40 and 0.55 (5 min^{-1}), respectively. As shown in Fig. 2B, the IC_{50} of the ACG_{12} effect on the activation of 1 nM G_{12} was $\approx 3\mu\text{M}$, which is identical to the IC_{50} observed with 10nM G_{12} . If one assumes that ACG_{12} inhibits G_{12} activation by acting on receptor sequences, the antagonizing effect of ACG_{12} on smaller amounts of G_{12} at a similar intensity of peptide 14 stimulation would be observed at lower concentrations of ACG_{12} , resulting in a lower IC_{50} . Conversely, if ACG_{12} acts on G_{12} , the potency of the ACG_{12} effect on smaller compared to larger amounts of G_{12} would not be altered, because the affinity of G_{12} for ACG_{12} would presumably determine the potency of ACG_{12} . Therefore, the present results suggest that ACG_{12} inhibits peptide 14/ G_{12} coupling by acting on G_{12} with constant affinity.

By examining the relationship between the structure of ACG_{12} and its function in the inhibition assay described above, it was possible to determine the primary structure essential for the inhibitory action of ACG_{12} . In the experiments shown in Figs. 3a-3c, G_{12} at 10 nM was incubated with 30 μM peptide 14 in the presence of various concentrations of ACG_{12} or one of its structural

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variants, and GTP γ S binding to G $_{12}$ was measured. The sequence of each peptide is indicated above the graphs (a bar indicates the same sequence as intact ACG $_{12}$). The 0% and the 100% increases, which correspond to the basal and peptide 14-stimulated fractional GTP γ S binding to G $_{12}$, were similar to those described in Fig. 1A. These groups can be practically classified according to the inhibition observed at 10 μ M. The high potency peptides of the first group (Fig. 3A) display $\geq 80\%$ inhibition at 10 μ M concentration; these peptides are the A $_2$ variant (DAVTAVIIKNNLKDC; SEQ ID NO: 21), in which the Asp at position five is replaced by Ala, and the A $_5$ variant (DAVTDVIIKNNLKAC; SEQ ID NO: 24), in which the Asp at position 14 is replaced by Ala. The second, low potency group (Fig. 3B) displays $\leq 30\%$ inhibition at 10 μ M, and is made up of the A $_1$ variant (AAVTDVIIKNNLKDC; SEQ ID NO: 20), in which the Asp at position 1 is replaced by Ala; the A $_3$ variant (DAVTDVIIKANLKDC; SEQ ID NO: 22), in which the Asn at position 10 is replaced with Ala; the p9 variant (DAVTDVIIK; SEQ ID NO: 25), in which the carboxy-terminal 6 residues of ACG $_{12}$ are deleted; and the del C variant (DAVTDVIIKNNLKD; SEQ ID NO: 16), in which the C-terminal Cys is deleted from ACG $_{12}$. The third, intermediate potency group (Fig. 3C) consists of the A $_4$ variant (DAVTDVIIKNALKDC; SEQ ID NO: 23), in which the Asn at position 11 is replaced by Ala; and the p11 variant (DVIIKNNLKDC; SEQ ID NO: 26), in which the amino-terminal 4 residues are deleted.

These results suggest that the extreme amino-terminal Asp, the Asn residues at positions 10 and 11, and the carboxy-terminal Cys are essential for the inhibitory activity of ACG $_{12}$, and that, since both the amino-terminal residue and the carboxy-terminal residue of ACG $_{12}$ are required, the segment of G $_{12}\alpha$ represented by ACG $_{12}$ corresponds to the minimal anticouplone domain of

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$G_{i2}\alpha$. In contrast, the Glu residues at positions 2 and 14 appear to be dispensable. One can conclude from this information that, contrary to the primary hypothesis used for the initial search that anionic residues on $G_{i2}\alpha$ would interact with the basic residues of peptide 14, all of the negatively charged residues are unlikely to be oriented facing the basic residues of peptide 14 or MIII, or at least that such a matchup of anionic and basic residues is not of critical importance at some of the sites. It remains unclear how the Cys³⁵² residue of ACG_{i2} plays a key role.

To determine whether ACG_{i2} uncouples G_{i2} from the signals of intact receptors as well as from the signals of their effector peptides, the effects of ACG_{i2} in mAChR- G_{i2} reconstituted phospholipid vesicles was studied (Fig. 4). In these vesicles, the basal value of rate constant k_{app} of GTPyS binding to G_{i2} in the absence of acetylcholine was 0.112 ± 0.005 (min⁻¹); 10 mM acetylcholine (for 20 min at 30°C) increased the k_{app} value to 0.286 ± 0.082 . The magnitude of the acetylcholine effect is similar to that previously reported (Kurose et al., J. Biol. Chem. 261:6423-6428, 1986). When 10 mM acetylcholine was incubated with the vesicles in the presence of 30 μ M ACG_{i2}, the k_{app} value of GTPyS binding to G_{i2} was found to be 0.157 ± 0.013 . This indicates that the G_{i2} activation induced by acetylcholine in AChR- G_{i2} vesicles was $\approx 75\%$ inhibited by ACG_{i2}. ACG_{i2}-induced inhibition was saturated at 30 μ M (data not shown). ACG_{i2} had no significant effect on basal G_{i2} activity in the absence of acetylcholine (Fig. 4).

A model illustrating the possible way in which ACG_{i2} blocks the interaction of G_{i2} and Peptide 14 is shown in Fig. 5. It is postulated that the anticouplone portion of $G_{i2}\alpha$ acts on a hypothetical second site of $G_{i2}\alpha$ itself, thereby stabilizing $G_{i2}\alpha$ in the unstimulated state

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(Fig. 5, left). Peptide 14, which has the sequence of the effector (couplone) region of IGF-IIR, binds to the contact site on $G_{i2}\alpha$, resulting in a conformational change in $G_{i2}\alpha$ that diminishes the accessibility of the anticouplone region of $G_{i2}\alpha$ to the hypothetical second site on $G_{i2}\alpha$; this leads to a decrease in binding of the two regions of $G_{i2}\alpha$ and a concomitant activation of the G protein (Fig. 5, upper right). Addition of ACG_{i2} peptide causes the hypothetical second site on $G_{i2}\alpha$ to be occupied regardless of the presence or absence of Peptide 14, thereby stabilizing $G_{i2}\alpha$ in the inactive state in a manner that is independent of (and so does not compete with) $G_{i2}\alpha$ binding to Peptide 14. Although the receptor-contact site is suggested to be different than the ACG_{i2} region of $G_{i2}\alpha$ in this figure, it should be emphasized that they are not necessarily different. Furthermore, it is noted that in the actual intracellular environment, there are other factors (e.g., nucleotide-binding status, GTP hydrolytic activity, and association with $G\beta\gamma$) that critically affect the equilibrium of each step. Because ACG_{i2} has different effects on the various G proteins (see above), it is unlikely that the hypothetical second site resides on $G\beta\gamma$ (which is identical for all of the G proteins) rather than $G\alpha$ (which is G-protein-specific).

25 Cell-based Assays

Inhibition of somatostatin receptor function. It is known that somatostatin induces K^+ channel activation in a manner sensitive to pertussis toxin in human growth hormone-secreting tumors (Yamashita et al., Am. J. Physiol. 253:E28-E32, 1987). The effect of each of ACG_{i2} , ACG_{i3} , ACG_s , ACG_{o1} , and ACG_{o2} on somatostatin-induced K^+ currents in human pituitary cells was measured using the patch clamp technique, as described below.

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Three human growth hormone-secreting pituitary adenomas were obtained at transsphenoidal surgery. The tissue was minced into small pieces (less than 1 mm in diameter) and was treated with 1000 units/ml dispase (Wako, Japan). These dissociated cells ($\approx 10^5$ cells/ml) were seeded onto 35-mm culture dishes. The monolayer cells were cultured at 37°C under 5% CO₂ in humidified air in Delbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated FCS.

Electrophysiological experiments were done within 4 weeks after cell preparation.

A whole cell variation of the patch electrode voltage clamp technique was used to record membrane currents (Hamill et al., Pflüger Arch. 391:85-100, 1987). The patch electrode solution contained 95 mM potassium aspartate, 47.5 mM KCl, 1 mM MgCl₂, 5.0 mM EGTA (potassium salt), 10 mM HEPES/KOH (pH 7.2), 2 mM ATP and 0.1 mM GTP. One of the antidiuretic peptides, dissolved in distilled water, was mixed with the patch electrode solution to a final concentration of 100 μ M, and was introduced into the interior of a pituitary cell in accordance with the published method. The extracellular medium, composed of 125 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2.5 mM CaCl₂, and 10 mM HEPES/NaOH (pH 7.4), was continuously perfused around the test cell during recording, using a peristaltic pump, and 10⁻⁷M somatostatin was introduced by changing the perfused medium. The liquid junction potential between the extracellular solution and the internal solution was directly measured using a 3 M KCl electrode as a reference. The value of the liquid junction potential (-8 mV) was corrected in each experiment. Glass capillaries of 1.5 mm diameter with a filament were used to fabricate an electrode for the whole cell clamp. They were coated with Sylgard and fire-polished before use.

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The patch electrode resistance used in these studies was in the range of 5 to 8 M Ω . The seal resistance was over several tens of G Ω . A List EPC-7 amplifier was used for the membrane current and potential. Application of voltage or current pulse, data acquisition, and analysis were done with a Gateway 2000 computer using the pCLAMP program (Axon Instruments, CA). All experiments were performed at room temperature (22-25°C).

The intracellular solution that filled the inside of the pipet portion of the apparatus contained one of the following antidiuretic peptides at 100 μ M: ACG_{i2}, ACG_{i3}, ACG_s, ACG_{o1}, or ACG_{o2}. The exterior of the cell was bathed with the extracellular solution containing 100 nM somatostatin while the outward K⁺ current was monitored. In the absence of antidiuretic peptides, somatostatin induces approximately 20 pA K⁺ current over baseline. This somatostatin-induced current level is not affected by the presence of 100 μ M ACG_{i3}, ACG_s, ACG_{o1}, or ACG_{o2}. ACG_{i2}, however, abolished the somatostatin-induced increase in K⁺ current, reducing the measured current to the baseline level observed in the absence of somatostatin. These data suggest that G_{i2}, but not G_{i3}, G_s, G_{o1}, or G_{o2}, is involved in the somatostatin receptor/K⁺ channel linkage, and that ACG_{i2} can specifically inhibit the ability of the somatostatin receptor to act on ion channels. Since this function of the somatostatin receptor on endocrine cells is related to the inhibition of hormone secretion by such cells, modulation of this receptor/G protein system by ACG_{i2} permits the control of hormone secretion from endocrine cells.

Inhibition of cellular growth. G_{i2} α has been shown to be an oncogene in certain human tumors (Lyons et al, Science 249:655-659, 1990). The effect of the

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peptide ACG₁₂ on the growth of CHO cells was measured as follows:

CHO cells were grown in Ham F12 medium (GIBCO) plus 10% fetal calf serum (FCS). Once they reached at least 80% confluency, the cells were incubated with Ham F12 plus 0.5% FCS for 24 hours. They were then incubated for an additional 24 hours with Ham F12 plus 10% FCS in the presence of BUdR and various concentrations of ACG₁₂. The cells were then fixed and the BUdR incorporated into their nuclei was labeled in accordance with the instructions included with the cell proliferation detection kit (RPN.210, Amersham). Fig. 6 shows the relative extent of BUdR incorporation into cells in the presence of no ACG₁₂ (top photograph), 10 μ M ACG₁₂ (center photograph), or 100 μ M ACG₁₂ (bottom photograph), wherein a labelled nucleus signifies a proliferating cell. It can be seen that FCS-induced proliferation of CHO cells is inhibited by ACG₁₂ in a dose-dependent fashion, with 10 μ M ACG₁₂ producing significant inhibition and 100 μ M inhibiting labelling almost completely. This suggests that ACG₁₂ may have value as an anti-growth or anti-cancer reagent.

Inhibition of TGF β -induced matrix formation. The secretion of matrix proteins (in particular, fibronectin) by kidney, lung and liver cells may lead to sclerosis of those organs. Transforming growth factor- β (TGF β), which stimulates the secretion of fibronectin, may therefore contribute to such sclerosis. Since the TGF β receptor is a G_o-coupled receptor, the ability of ACG₁₂ to inhibit the TGF β -induced stimulation of fibronectin secretion by NRK (rat renal fibroblast) cells (Ignotz and Massagué, J. Biol. Chem. 261:4337-4345, 1986) was assayed.

NRK cells were cultured in RPMI 1640 supplemented with 5% FCS in a CO₂-incubator at 37°C. The medium was replaced every two days. Cells were regularly

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subcultured by trypsin-EDTA before reaching confluency. In the experiments described below, the cells were grown in monolayer to confluency in 6-well culture plates (Corning, NY), then incubated for 12 hours with RPMI 1640 plus 0.5% FCS, and for a further 12 hours with RPMI 1640 plus 0.5% FCS in the presence or absence of 50 pM TGF β (R & D System, Minneapolis, MN), to which was added 100 μ M ACG_{i2}, ACG_s, ACG_{o2}, or ACG_q. The medium from each well was collected and frozen at -20°C until assay.

10 Fibronectin secreted into each sample of medium was assessed by immunoblot as follows:

Each medium sample was mixed with an equal volume of 2x concentrated Laemmli buffer (Laemmli, Nature 227:680-685, 1970), and boiled at 90°C for 5 min.

15 Proteins were separated by electrophoresis on 7.5% SDS-polyacrylamide gel, and then transferred to a polyvinylidene difluoride sheet (Immobilon-P, Millipore) by electroelution at 0-4°C with constant voltage of 75 V for 8 hours. After transfer, the sheet was incubated

20 with PBS containing 5% bovine serum albumin and 2% skim milk (Defco) overnight at room temperature to block nonspecific protein binding. The sheet was then incubated with PBS containing 0.1% bovine serum albumin and an anti-fibronectin antibody (Chemicon International, CA) at a final concentration of 0.5 mg/ml for 3 hours at

25 room temperature. After washing with ice-cold PBS/0.05% Tween 20 three times, the sheet was incubated with 0.04 units/ml of a horseradish peroxidase-conjugated anti-rabbit IgG (Sigma, A-8257) in PBS/0.1% bovine serum

30 albumin for 3 hours at room temperature. The sheet was washed six times with ice-cold PBS/0.05% Tween 20, incubated with ECL (Enhanced ChemiLuminescence) solution (Amersham, RPN 2101) according to instructions for 1 min, and exposed on Kodak XAR5 film for 40-60 seconds. As

35 shown in Fig. 7, a significant amount of fibronectin is

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secreted by the cells exposed to $TGF\beta$, whether or not any of ACG_{i2} , ACG_s , or ACG_q is present in the culture medium. In contrast, those cells exposed to $TGF\beta$ in the presence of ACG_{o2} secrete relatively little fibronectin. This indicates that ACG_{o2} can inhibit the $TGF\beta$ -induced secretion of fibronectin by these cells, suggesting that ACG_{o2} has use as a therapeutic for the inhibition of organosclerosis, as well as of $TGF\beta$ activity in general.

Inhibition of T cell receptor function with ACG_q .

10 T cell receptor stimulation by monoclonal antibody OKT^3 in Jurkat cells, a line of human T cells, induces measurable polyphosphoinositide (PI) turnover. 10^7 cells were labelled with $5\mu Ci/ml$ [3H]myoinositol in inositol-free RPMI1640 for 12 hours. After incubating the cells
15 with $500\mu M$ ACG_q , samples containing 5×10^5 cells were then exposed to the monoclonal antibody OKT_3 (Ortho) at 1:100 dilution for 5 min. Perchloric acid was added to a final concentration of 10% to lyse the cells and terminate the reaction. Cell lysates were collected into tubes and
20 brought to pH 8 by the addition of KOH. Following centrifugation of the lysates, the supernatants were passed over a DOWX anion exchange column (Muromachi Chemicals, Japan) to separate inositol polyphosphates (IPs) from other inositol metabolites, and the amount of
25 [3H] label in the inositol trisphosphate (IP_3) fraction assessed. Cells exposed to OKT_3 incorporated [3H] into IP to a level 157% that of the control cells (cells not exposed to OKT_3), reflecting the stimulatory effect OKT_3 has on PI turnover in T cells. When the cells pretreated
30 with $500\mu M$ ACG_q were stimulated with OKT_3 , the level of [3H] incorporation into IP_3 was only 112% that of the unstimulated control cells; in contrast, pretreatment with $500\mu M$ ACG_{i2} did not inhibit stimulation of the cells by OKT_3 . These results indicate that ACG_q can block the

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function of the T cell receptor, suggesting a use of this anticouplone peptide as an immunosuppressant.

In vivo Assay of Anticouplone Peptides

Myopathic syrian hamsters BIO 53.58 spontaneously
5 develop fibrosis of the heart muscle (i.e., development
of extraneous collagen fibers that eventually interfere
with muscle function) by about 16 weeks of age, and as
such are used as models for the human disease
cardiomyopathy. In the experiment reported herein, 10-
10 week-old BIO 53.58 hamsters received intraperitoneal
injections with 5 μ l of 10 mM ACG_q, 10 mM ACG_{i2}, or
physiological saline once a week for 6 weeks. The
animals were then decapitated and their hearts were
excised, fixed in 10% formaldehyde solution, and cut
15 horizontally at the level of the papillary muscle to make
sections for light microscopy. The muscle sections were
stained with sirius red (which specifically stains
collagen), and evaluated under the microscope. In each
photomicrograph shown in Fig. 8, the right ventricular
20 free wall is located at the left of the photomicrograph
and the interventricular septum is on the right. As
shown in Fig. 8, top panel, the control hamsters
exhibited a significant amount of heart muscle fibrosis
(indicated by darkly stained lines). In contrast, the
25 animals treated with ACG_{i2} for six weeks had remarkably
diminished collagen fiber formation (Fig. 8, bottom
panel). Hamsters which received ACG_q had undiminished,
control levels of fibrosis (data not shown).

Use

30 Each of the anticouplone peptides of the invention
may be used as a therapeutic for appropriate indications.
ACG_{i2} may be used where inhibition of G_{i1}- or G_{i2}-coupled
receptor function is desired: for example, to treat

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neuromuscular diseases and cardiac arrhythmia by inhibiting muscarinic acetylcholine receptors; to alleviate dwarfism by inhibiting stimulation of somatostatin receptors on pituitary cells; to block
5 cancerous cell proliferation (or other undesired cell growth, such as cardiomyopathy) by inhibiting the G_{i2} oncogene; to treat organofibrosis by inhibiting $TGF\beta$ receptors; and to treat malignant hypercalcemia by inhibiting the parathyroid/parathyroid-related protein
10 (PTH/PTHrp) receptor. ACG_{i3} would be useful for treatment of conditions related to organization and polarization of cells and cellular structures, as during organ development.

ACG_{o1} will inhibit stimulation of the nerve growth
15 factor receptor (NGFR), the activity of which is thought to affect neuronal growth. ACG_{o2} is useful for inhibiting the $TGF\beta$ -induced stimulation of fibronectin secretion which leads to fibrosis and sclerosis of organs such as kidney, lung and liver. Inhibition of $TGF\beta$ is also
20 useful in controlling the generation of cancer clones and in treating glomeronephritis. In addition, ACG_{o2} can be used to prevent cancer cell growth attributable to abnormal stimulation of the cells by a mutant G_{o2} oncogene.

25 Because G_q is thought to be the G protein to which the T cell receptor is coupled, ACG_q would be useful for inhibiting the function of this receptor, and thus the activation of T cells. This anticoupling therefore may be used as an immunosuppressant, such as in organ and
30 bone marrow transplant recipients, and to treat autoimmune disease. ACG_q also has potential as a treatment for AIDS, by inhibiting the abnormal signalling that results from binding of HIV gp120 to CD4 on the T cell surface. In addition, ACG_q , which inhibits

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signalling through the angiotensin receptor, may be used as a treatment for hypertension.

G_s -coupled receptors include the glucagon receptor, the β_2 AR, and the PTH/PTHrP receptor. ACG_s could therefore be utilized as a treatment for diabetes, hypertension, and malignant hypercalcemia. Because a mutant form of G_s acts constitutively in some pituitary tumors, ACG_s could be used to treat such pituitary tumor-related conditions as gigantism and secondary menopause.

10 The treatment protocol to be used for any one of the above indications could be readily determined by one of ordinary skill in the art of pharmacology, given the teachings set forth above. The peptides of the invention may be administered by a standard method such as

15 intravenous, intraperitoneal, intramuscular, or subcutaneous injection, and may be administered in a single dose, several doses spread over time, or a continuous infusion. The dosage will vary with the age and condition of the patient and the particular condition

20 being treated, but is expected to be in the range of 0.5 μ g/kg/day to 50 mg/kg/day. A dosage which results in a concentration at the target site of approximately 10-100 μ M would be an effective dose consistent with the experimental results provided above. Incorporating the

25 peptides into micelles or liposomes may improve delivery into cells.

Other Embodiments

Other embodiments are within the claims set forth below. For instance, many other receptors are known or

30 believed to be G-coupled receptors, based in part on the their distinctive arrangement of multiple transmembrane domains known to be typical of G-coupled receptors; inappropriate signalling through these receptors can be controlled by the peptides of the invention. An example

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is the thyroid-stimulating hormone (TSH) receptor, overstimulation of which (e.g., by a receptor-specific antibody) leads to hyperthyroidism, or Basedow's disease. This receptor is probably a G_s -coupled receptor. Another
5 example is the G protein-coupled insulin receptor, which may be overstimulated in a patient with an insulin-secreting tumor.

As described above, some amino acid residues within the defined anticouplone sequences can be replaced
10 with other residues, with no significant loss of biological activity. For example, in ACG_{12} , an Asp→Ala substitution at position 5 or 14 may be made with little or no loss of activity, while similar nonconservative substitutions at positions 1, 10, and 11 resulted in a
15 significant loss of activity in the *in vitro* assay used. In general, conservative substitutions in which the relative charge of the residue occupying the substituted position does not vary (i.e., replacing an anionic residue with another anionic residue, or a hydrophobic
20 residue with another hydrophobic residue, etc.) will have less effect on the biological activity of the resulting peptide than will nonconservative substitutions. While deletion of some residues may well not affect biological activity significantly, it is known from the work
25 described above that the residues in both the first and the last (15th) positions of ACG_{12} are important for activity of that anticouplone. Variants of the anticouplone peptides utilized in the above experiments can be readily made by one of ordinary skill in the art
30 of peptide synthesis, using standard methods of automated synthesis; each such peptide can then be tested in one or more of the biological assays described above. The *in vitro*, cell-free assays are particularly suited to
testing large numbers of such peptides quickly and
35 efficiently, permitting one to produce and evaluate

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hundreds of peptide variants without undue experimentation. Thus, the invention is meant to include any peptide which (a) has at least 70% sequence identity with one of the anticouplone peptides described above
5 (preferably at least 85%), and (b) has at least 80% of the biological activity of the anticouplone peptide which it most closely resembles, in any of the biological assays described above in which that anticouplone peptide has been shown to be active. Of course, a peptide which
10 includes the sequence of one of the anticouplones described above, plus additional sequence (including but not limited to sequence derived from the G-protein of which the anticouplone is a part), would be expected to have undiminished biological activity compared to the
15 anticouplone itself, provided that the peptide is not too bulky or too highly charged to pass through the cellular membrane as readily as does the anticouplone peptide. Again, such longer peptides can be easily prepared and tested as described. Examples of such longer peptides
20 are presented above as "alternative" anticouplone peptides, shown as SEQ ID NOs: 4, 6, 9, 11, 13, and 15.

Also within the invention are analogs of the above peptides, in which one or more peptide bonds have been replaced with an alternative type of covalent bond (a
25 "peptide mimetic") which is not susceptible to cleavage by peptidases. While the *in vivo* results described above suggest that proteolytic degradation of the peptides following injection into the subject animal may not be a problem, it may be that for certain of the peptides,
30 replacement of a particularly sensitive peptide bond with a noncleavable peptide mimetic will make the resulting compound more stable and thus more useful as a therapeutic. Such mimetics, and methods of incorporating them into polypeptides, are well known in the art.
35 Similarly, the replacement of an L-amino acid residue of

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the anticouplone peptide with the corresponding D-amino acid residue is a standard way of rendering the polypeptide less sensitive to proteolysis. Also useful in this regard are amino-terminal blocking groups such as

5 t-butyloxycarbonyl, acetyl, ethyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4-dinitrophenyl. Blocking the charged amino- and carboxy-

10 termini of the peptides would have the additional benefit of enhancing passage of the peptide through the hydrophobic cellular membrane and into the cell.

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SEQUENCE LISTING**(1) GENERAL INFORMATION:**

- (i) APPLICANT: Nishimoto, Ikuo
- (ii) TITLE OF INVENTION: REGULATOR REGIONS OF G PROTEINS
- (iii) NUMBER OF SEQUENCES: 34
- (iv) CORRESPONDENCE ADDRESS:
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- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: MS-DOS (Version 5.0)
(D) SOFTWARE: WordPerfect (Version 5.1)
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/019,073
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- (viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

- 31 -

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asp Xaa Xaa Xaa Xaa Val Ile Ile Lys Asn Asn Leu Xaa Xaa Cys
 1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asp Ala Val Thr Xaa Val Ile Ile Lys Asn Asn Leu Lys Xaa Cys
 1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
 1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Asp Thr Lys Asn Val Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile
 1 5 10 15

Ile Lys Asn Asn Leu Lys Asp Cys
 20

- 32 -

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Glu Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Asp Thr Lys Asn Val Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile
1 5 10 15
Ile Lys Asn Asn Leu Lys Glu Cys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asp Ala Val Thr Xaa Ile Ile Ile Ala Xaa Asn Leu Arg Xaa Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

- 33 -

Asp Ala Val Thr Asp Ile Ile Ile Ala Asn Asn Leu Arg Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Asn Asn Ile Gln Val Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala
1 5 10 15

Asn Asn Leu Arg Gly Cys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Asp Ala Val Thr Asp Ile Ile Ile Ala Lys Asn Leu Arg Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Asn Asn Ile Gln Phe Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala
1 5 10 15

Lys Asn Leu Arg Gly Cys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid

- 34 -

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asn Asp Cys Arg Asp Ile Ile Gln Arg Met His Leu Arg Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Asp Thr Glu Asn Ile Arg Arg Val Phe Asn Asp Cys Arg Asp Ile Ile
1 5 10 15
Gln Arg Met His Leu Arg Gln Tyr
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Asp Thr Glu Asn Ile Arg Phe Val Phe Ala Ala Val Lys Asp Thr Ile
1 5 10 15
Leu Gln Leu Asn Leu Lys Glu Tyr
20

- 35 -

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Glu
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Glu Ala Ala Ser Tyr Ile Gln Ser Lys Phe Glu Lys Leu Asn
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Lys Asn Asn Leu Lys Asp Cys Gly Leu Phe
1 5 10

- 36 -

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Ala Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Asp Ala Val Thr Ala Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Asp Ala Val Thr Asp Val Ile Ile Lys Ala Asn Leu Lys Asp Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Ala Leu Lys Asp Cys
1 5 10 15

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Ala Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Asp Ala Val Thr Asp Val Ile Ile Lys
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Arg Val Gly Leu Val Arg Gly Glu Lys Ala Arg Lys Gly Lys
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 28:

(i) SEQUENCE CHARACTERISTICS:

- 38 -

(A) LENGTH: 25
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Leu Thr Ala Cys Leu Leu Thr Leu Leu Leu Tyr Arg Val Gly Leu Val
1 5 10 15
Arg Gly Glu Lys Ala Arg Lys Gly Lys
20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Arg Arg Ser Ser Lys Phe Cys Leu Lys Glu His Lys Ala Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Arg Asn Gln Val Arg Lys Lys Arg Gln Met Ala Ala Arg Glu Arg Lys
1 5 10 15
Val Thr Arg

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Xaa Cys
1 5 10 15

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Asp Thr Lys Asn Val Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile
1 5 10 15
Ile Lys Asn Asn Leu Lys Xaa Cys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Asp Ala Val Thr Asp Ile Ile Ile Ala Xaa Asn Leu Arg Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Asn Asn Ile Gln Xaa Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala
1 5 10 15
Xaa Asn Leu Arg Gly Cys
20

What is claimed is:

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CLAIMS

1. A molecule comprising the anticouplone sequence of a G protein, wherein said molecule contains no more than 50 amino acid residues.
2. The molecule of claim 1, wherein said molecule
5 contains no more than 30 amino acid residues.
3. The molecule of claim 1, wherein said G protein is a mammalian G protein.
4. The molecule of claim 2, wherein said molecule
is a peptide the amino acid sequence of which is
10 essentially identical to said anticouplone.
5. A molecule comprising the amino acid sequence
DXXXXVIIKNNLXXC (SEQ ID NO: 1),
wherein said molecule contains no more than 40 amino acid
residues.
- 15 6. The molecule of claim 5, wherein said sequence
is
DAVTXVIIKNNLKXC (SEQ ID NO: 2).
7. The molecule of claim 6, wherein said molecule
comprises the amino acid sequence
20 DAVTDVIIKNNLKDC (SEQ ID NO: 3).
8. The molecule of claim 6, wherein said molecule
comprises the amino acid sequence
DTKNVQFVFDVTDVIIKNNLKDC (SEQ ID NO: 4).
9. The molecule of claim 5, wherein said molecule
25 comprises the amino acid sequence
DAVTDVIIKNNLKEC (SEQ ID NO: 5).

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10. The molecule of claim 9, wherein said molecule comprises the amino acid sequence
DTKNVQFVFDAVTDVVIKNNLKEC (SEQ ID NO: 6).

5 11. A molecule comprising the amino acid sequence
DAVTXIIIAXNLRXC (SEQ ID NO: 7),
wherein said molecule contains no more than 40 amino acid residues.

12. The molecule of claim 11, wherein said molecule comprises the amino acid sequence
10 DAVTDIIIANNLRGC (SEQ ID NO: 8).

13. The molecule of claim 11, wherein said molecule comprises the amino acid sequence
NNIQVVFDAVTDIIIANNLRGC (SEQ ID NO: 9).

14. The molecule of claim 11, wherein said
15 molecule comprises the amino acid sequence
DAVTDIIIAKNLRGC (SEQ ID NO: 10).

15. The molecule of claim 14, wherein said molecule comprises the amino acid sequence
NNIQVFVDAVTDIIIAKNLRGC (SEQ ID NO: 11).

20 16. A molecule comprising the amino acid sequence
NDCRDIIQRMHLRQY (SEQ ID NO: 12),
wherein said molecule contains no more than 40 amino acid residues.

17. The molecule of claim 16, wherein said
25 molecule comprises the amino acid sequence
DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13).

18. A molecule comprising the amino acid sequence

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AAVKDTILQLNLKEY (SEQ ID NO: 14),
wherein said molecule contains no more than 40 amino acid
residues.

19. The molecule of claim 18, wherein said
5 molecule comprises the amino acid sequence
DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).

20. A method of inhibiting activation of a G
protein by a G-coupled receptor on a cell, said method
comprising contacting said cell with a molecule
10 comprising the anticouplone sequence of said G protein,
said molecule containing no more than 40 amino acid
residues.

21. The method of claim 20, wherein said G
protein is G_{i1} , G_{i2} , or G_{i3} and said anticouplone sequence
15 is

DAVTDVVIKNNLKXC (SEQ ID NO: 31) or
DTKNVQFVFDVTDVVIKNNLKXC (SEQ ID NO: 32).

22. The method of claim 21, wherein said G-
coupled receptor is insulin-like growth factor II
20 receptor (IGF-IIR), muscarinic acetylcholine receptor
(mAChR), somatostatin receptor, D_2 -dopamine receptor, α_2 -
adrenergic receptor, adenosine receptor, thrombin
receptor, or TGF β receptor.

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23. The method of claim 20, wherein said G protein is G_{O1} or G_{O2} and said anticouplone sequence is
DAVTDIIIAXNLRGC (SEQ ID NO: 33) or
NNIQXVFDAVTDIIIAXNLRGC (SEQ ID NO: 34).

5 24. The method of claim 23, wherein said G-coupled receptor is transforming growth factor- β receptor (TGF β R), γ -butyric acid (GABA) receptor, somatostatin receptor, muscarinic acetylcholine receptor, adenosine receptor, thrombin receptor, or α_2 -adrenergic receptor.

10 25. The method of claim 20, wherein said G protein is G_s and said anticouplone sequence is
NDCRDIIQRMHLRQY (SEQ ID NO: 12) or
DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13).

15 26. The method of claim 25, wherein said G-coupled receptor is β_2 -adrenergic receptor, glucagon receptor, or D_1 -dopamine receptor.

 27. The method of claim 20, wherein said G protein is G_q and said anticouplone sequence is
AAVKDTILQLNLKEY (SEQ ID NO: 14) or
20 DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).

 28. The method of claim 27, wherein said G-coupled receptor is the T cell receptor, PTH/PTHrP receptor, calcitonin receptor, endothelin receptor, angiotensin receptor, platelet activating factor
25 receptor, or thromboxane A_2 receptor.

1 / 8

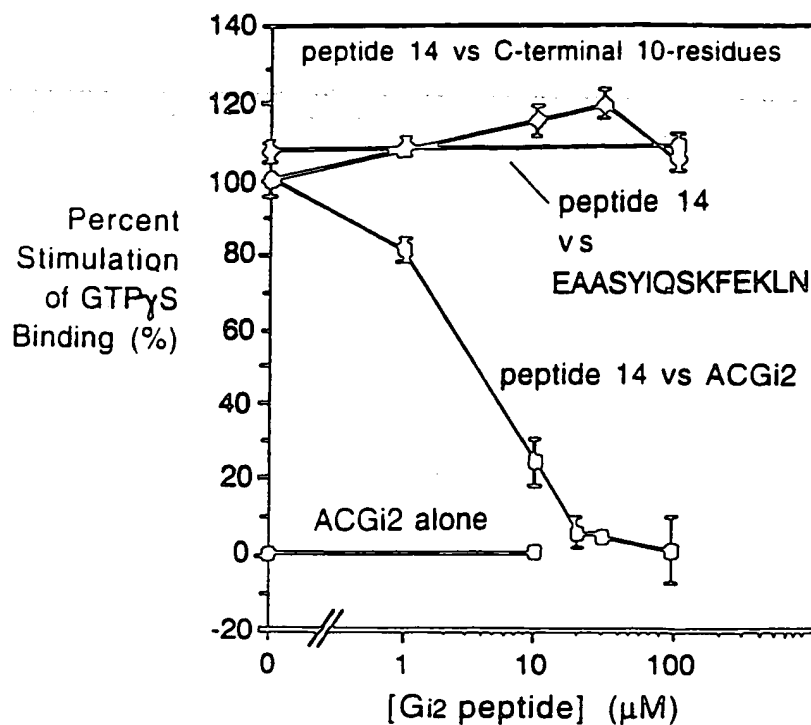


FIG. 1A

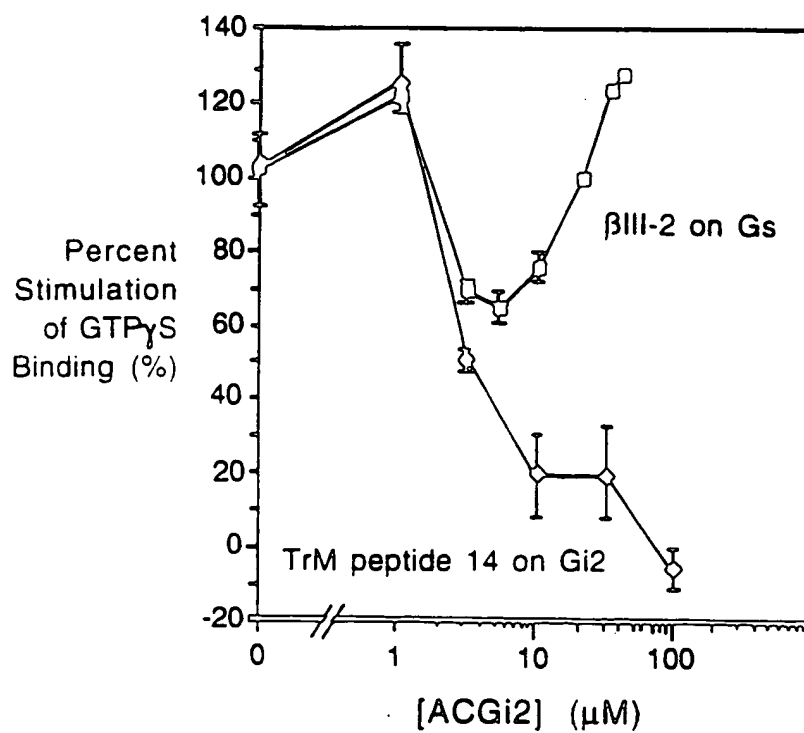
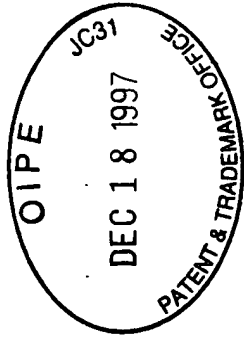


FIG. 1B



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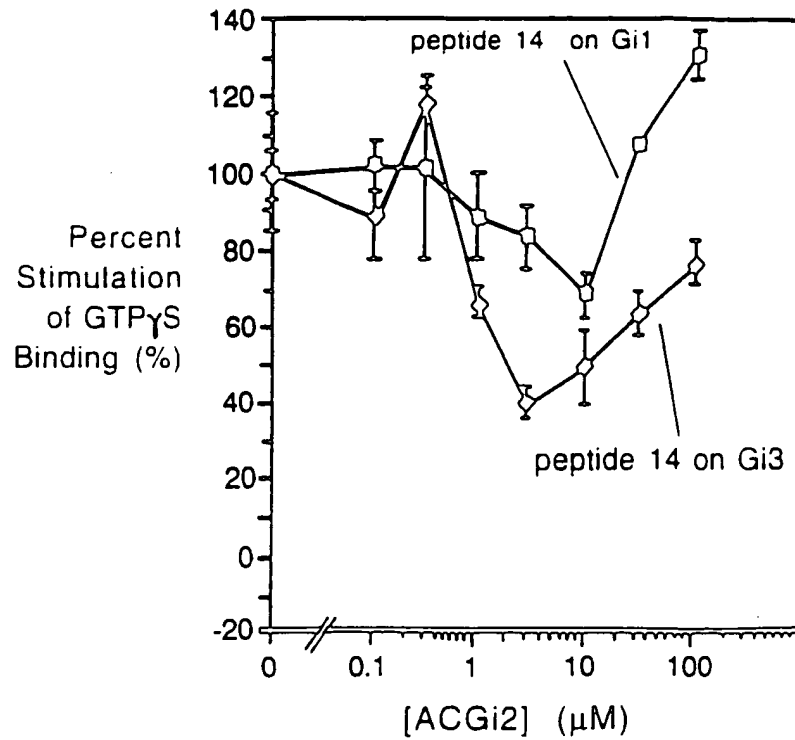


FIG. 1C

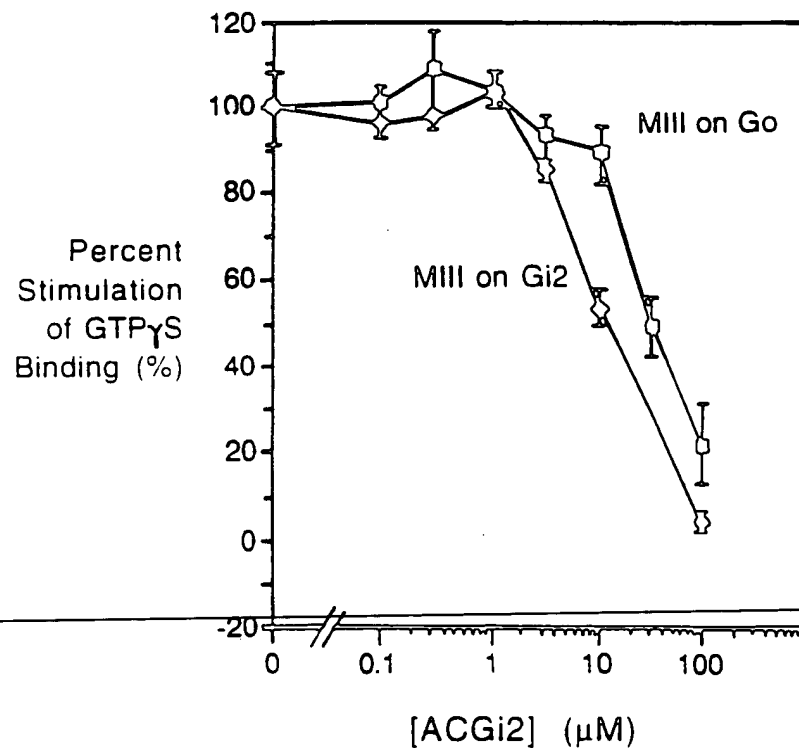


FIG. 1D

SUBSTITUTE SHEET (RULE 26)

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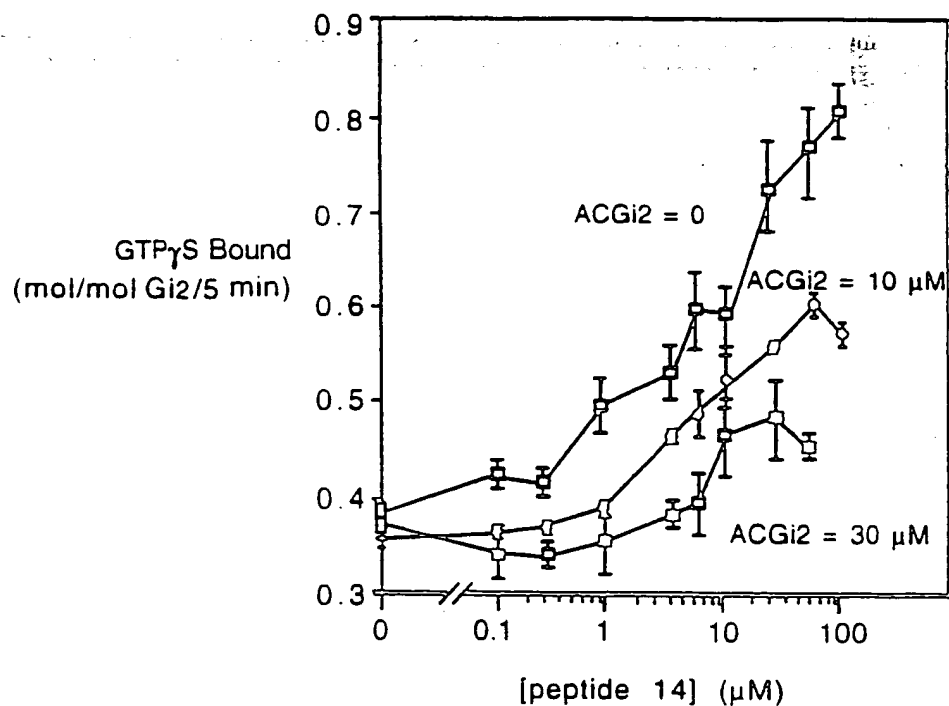


FIG. 2A

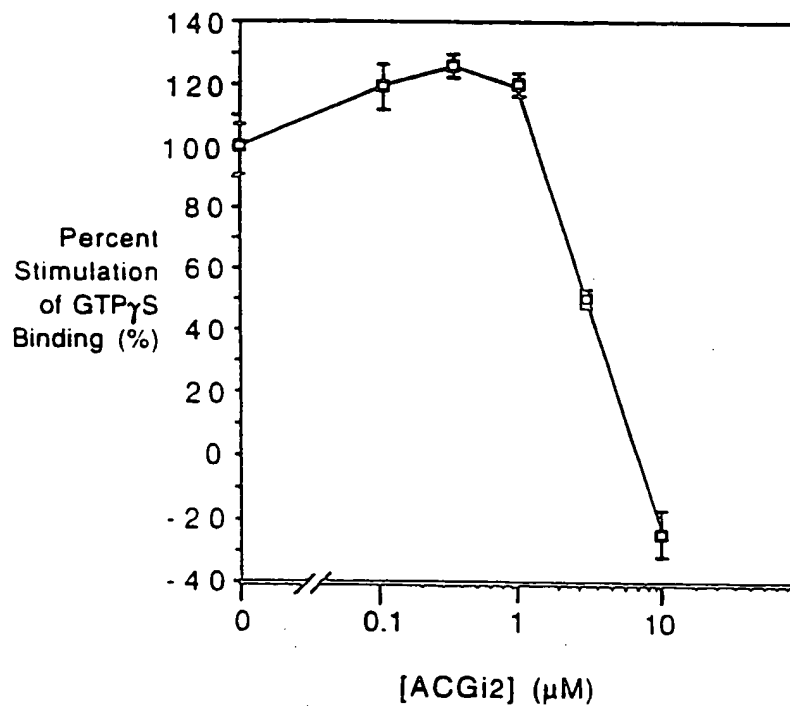


FIG. 2B

SUBSTITUTE SHEET (RULE 26)

high potency group

intact DAVTDVIKNNLKDC
A2 — A —
A5 — A —

low potency group

intact DAVTDVIKNNLKDC
A1 A —
A3 — A —
P9 —
del C —

intermediate group

intact DAVTDVIKNNLKDC
A4 —
P11 —

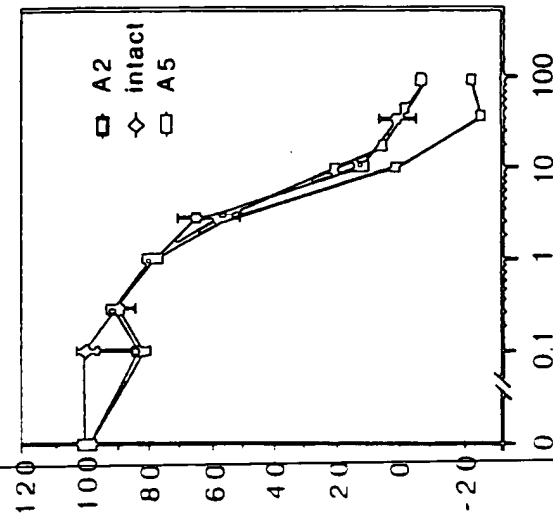


FIG. 3A

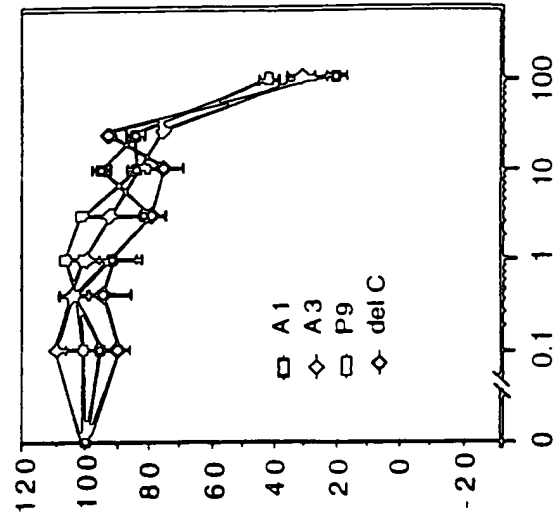


FIG. 3B

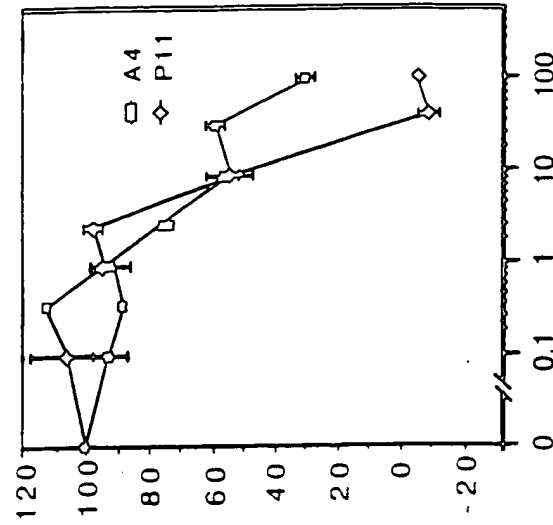


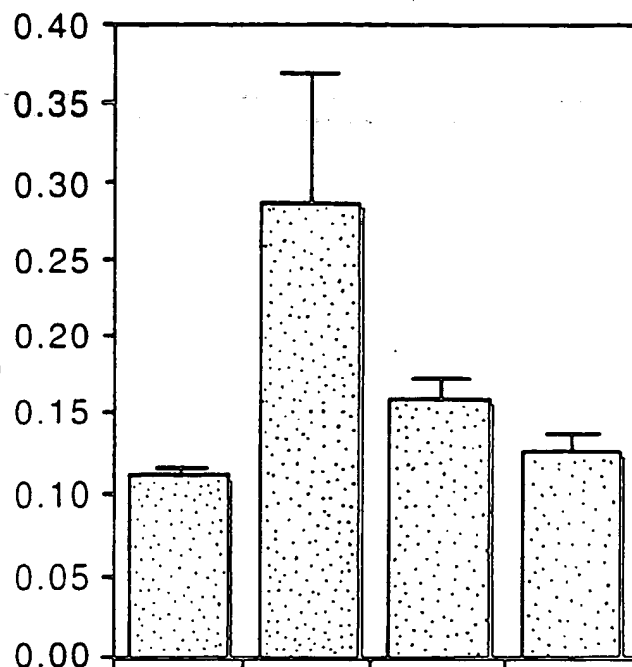
FIG. 3C

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FIG. 4

Rate Constant
of
GTP γ S Binding (min⁻¹)



ACh (10 mM)	-	+	+	-
ACGi2 (30 μM)	-	-	+	+

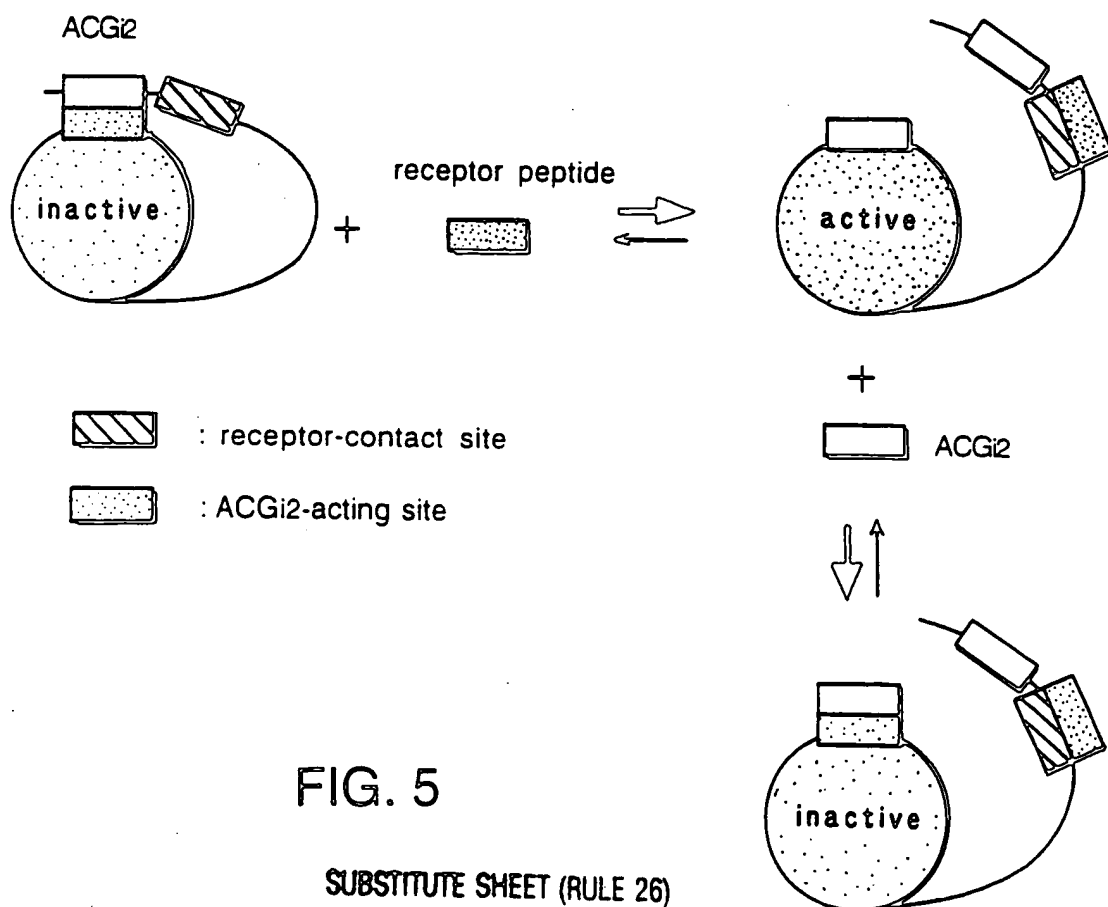
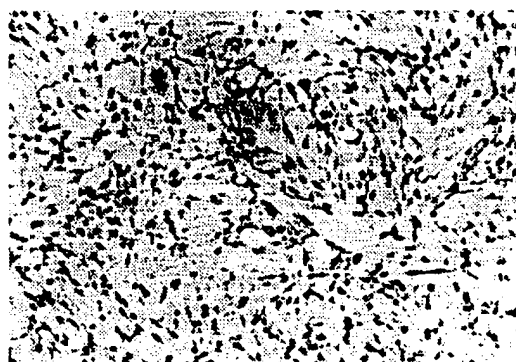
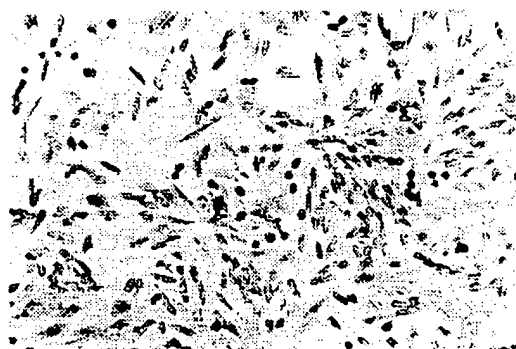


FIG. 5

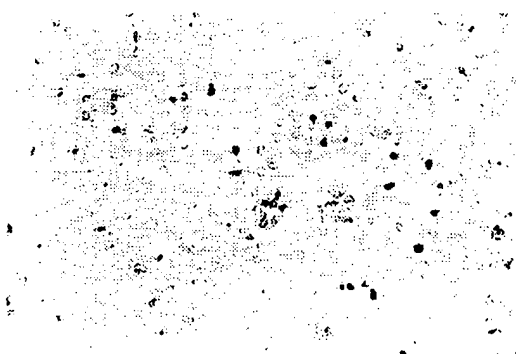
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0 μ M
ACGi2



10 μ M
ACGi2



100 μ M
ACGi2

FIG. 6

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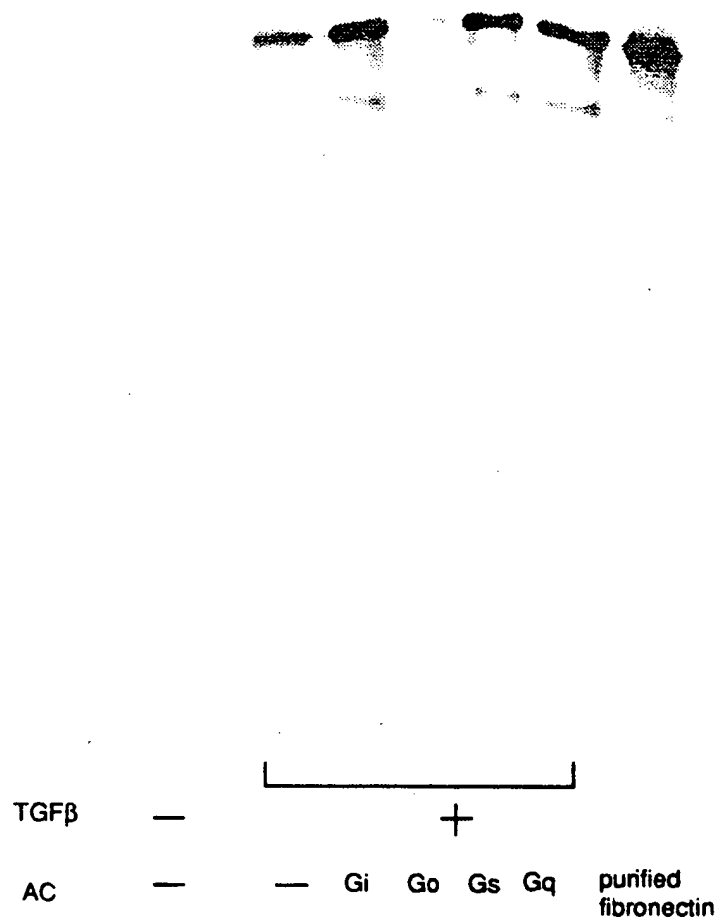
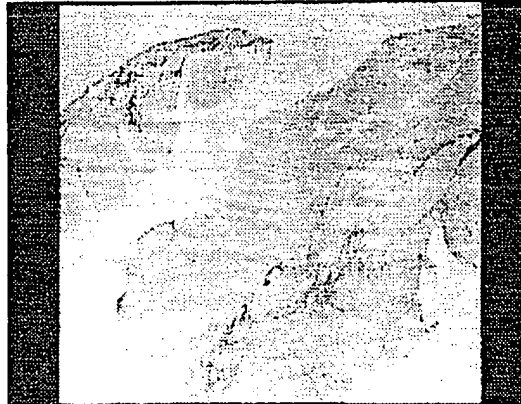


FIG. 7

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Control



+ ACGi2

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/01768

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 37/02; C07K 7/08, 7/10

US CL : 530/324, 325, 326

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, CAS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, Vol. 249, issued 10 August 1990, Lyons et al , "Two G Protein Oncogenes in Human Endocrine Tumors", pp. 628-631.	1-28
A	Journal of Biological Chemistry, Vol. 264, No. 24, issued 25 August 1989, Nishimoto, et al , "Possible Direct Linkage of Insulin-like Growth Factor-II Receptor with Guanine Nucleotide-Binding Proteins", pp 14029-14038.	1-28

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention
* A* document defining the general state of the art which is not considered to be part of particular relevance	* X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* A*	document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means		
* P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

24 MAY 1994

Date of mailing of the international search report

JUN 17 1994

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Box PCT
Washington, D.C. 20231

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